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A. Background:

The plant hormone auxin (indole-3-acetic acid, IAA) plays a critical role in the regulation of cell division, growth, and differentiation, as well as in mediating plant responses to environmental cues such as gravity, light, and wounding. Although the molecular mechanism of auxin action is still unknown, it is likely that auxin initiates cellular responses by binding to specific receptor proteins. The objective of my research is to understand the role of auxin-specific plasma membrane (PM), receptors in plant growth development.

Clearly, the isolation of auxin-specific receptors is an important step toward the molecular dissection of auxin action. In theory, such receptors are the first step in linking auxin-inducible genes and/or other events in the signal transduction pathway. Previous efforts to isolate and identify specific auxin receptors have been hampered by two distinct problems. First, the relatively weak association of IAA with putative receptors makes the isolation and characterization of such receptors difficult. Second, proof of a connection between putative receptors and auxin action has been difficult to obtain. This in turn makes it difficult to separate fortuitous binding from physiologically meaningful ligand-receptor interactions. I believe that work in my laboratory and elsewhere has not progressed to the point that both of these problems have or can be solved. Ultimately the isolation and characterization of auxin receptors will impact efforts to manipulate the growth and yield of plants on earth and in the microgravity environment in space.

B. Recent Progress Under Project NAG-1849:

We have isolated and identified putative auxin receptors or auxin binding proteins (ABPs) by the preparation of highly enriched PM vesicles by aqueous two-phase partitioning, followed by photoaffinity labeling of polypeptides in these vesicles using the auxin analogue ^3H -5N₃-IAA (azido-IAA). We have succeeded in eliminating nonspecific azido-IAA labeling by conducting the photolysis reaction at -196° C. Under these conditions, photolysis of azido-IAA in the presence of membrane proteins from auxin responsive tissues of a variety of species results in the high specific-activity labeling of a low abundance 40-42 kDa polypeptide doublet which displays properties consistent with those expected for an auxin receptor.

In an attempt to determine if the azido-IAA labeled polypeptides are indeed involved in auxin responsiveness, we examined an auxin non-responsive mutant

of tomato (known as diageotropica (dgt)). This plant is a single gene recessive mutant of the tomato variety VFN8. Membranes from roots and hypocotyls of the wild-type tomato, VFN8, contain the characteristic two polypeptides (40-42 kDa) which are labeled with high specific activity by azido-IAA. In contrast, membrane proteins from *dgt* hypocotyls are only very slightly labeled relative to similar preparations from VFN8 hypocotyls. This is an exciting finding in that it identifies a protein which is missing or altered in only the mutant line of tomato and that protein is an auxin binding protein. Since the *dgt* mutation appears to involve auxin perception, this provides not only confirmation of the probable receptor identity of the 40-42 kDa polypeptides, but also associates that receptor with a specific set of morphological and physiological attributes which are known to be connected to auxin.

C. Publications Supported by the Two Years of Funding:

Rayle, D.L. Calcium bridges are not load-bearing cell wall bonds in Avena coleoptiles. *Planta* 178:92-95 (1989).

Hicks, G.R., D.L. Rayle, A.M. Jones, and T.L. Lomax. Specific photoaffinity labeling of two plasma membrane polypeptides with an azido auxin. *Proc. Natl. Acad. Sci.* 86:4948-4952 (1989).

Hicks, G.R., D.L. Rayle, and T.L. Lomax. The diageotropica mutant of tomato lacks high specific activity auxin binding sites. *Science* 245:52-54 (1989).

Migliaccio, R., and D.L. Rayle. Effects of asymmetric auxin application on Helianthus hypocotyl curvature. *Plant Physiol.* 91:466-468 (1989).

Daniel, S.G., D.L. Rayle, and R.E. Cleland. Auxin physiology of the tomato mutant, diageotropica. *Plant Physiol.* 91:804-807 (1989).

Gibeaut, D.M., N. Karuppiyah, S.R. Chang, T.G. Brock, B. Badlamui, K.C. Lu, D. Kim, N.S. Gshosheh, D.L. Rayle, N.C. Carpita, and P.B. Kaufman. Cell wall and enzyme changes during the graviresponse of the leaf-sheath pulvinus in oat. *Plant Physiol.* 94:411-416 (1990).

Rayle, D.L., S. Nowbar, and R.E. Cleland. The epidermis of the pea epicotyl is not a unique target tissue for auxin-induced growth. *Plant Physiol.* 97:449-451 (1991).